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*Published in:*  
 PLoS ONE

*DOI:*  
 [10.1371/journal.pone.0171545](https://doi.org/10.1371/journal.pone.0171545)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
 Publisher's PDF, also known as Version of record

*Publication date:*  
 2017

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Siland, J. E., Geelhoed, B., van Gelder, I. C., van der Harst, P., & Rienstra, M. (2017). Telomere length and incident atrial fibrillation: data of the PREVEND cohort. *PLoS ONE*, 12(2), [e0171545].  
 <https://doi.org/10.1371/journal.pone.0171545>

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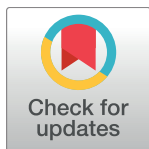
RESEARCH ARTICLE

# Telomere length and incident atrial fibrillation – data of the PREVEND cohort

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## Abstract

### Background

The incidence of atrial fibrillation (AF) increases with age. Telomere length is considered a marker of biological ageing. We investigated the association between leukocyte telomere length and incident AF in the Dutch Prevention of Renal and Vascular End-stage Disease (PREVEND) study.

### Methods

We included 7775 individuals without prevalent AF, and with leukocyte telomere length measured. Mean telomere length was determined by a monochrome multiplex quantitative polymerase chain reaction-based assay.

### Results

Mean age of our cohort was  $49 \pm 13$  years, and 50% were men. During a mean follow-up of  $11.4 \pm 2.9$  years incident AF was detected in 367 (4.7%) individuals. Telomere length was shorter in individuals developing incident AF compared to those without AF ( $p = 0.013$ ). Incident AF was inversely related to the telomere length. In the quartile with the longest telomere length 68 (3.5%) individuals developed AF, in the shortest telomere length quartile 100 (5.1%) individuals ( $p = 0.032$ ). Telomere length was associated with incident AF in the second shortest telomere length quartile using the longest telomere length quartile as reference (hazard ratio 1.64; 95% CI 1.02–2.66;  $p = 0.043$ ). After including age or AF risk factors, the relation between telomere length and incident AF was no longer significant. We found a significant interaction of age, male sex, systolic blood pressure, BMI, heart failure, and myocardial infarction with telomere length for the association with incident AF.

### Conclusions

We found that shorter leukocyte telomere length is not independently associated with incident AF in a community-based cohort.

## OPEN ACCESS

**Citation:** Siland JE, Geelhoed B, van Gelder IC, van der Harst P, Rienstra M (2017) Telomere length and incident atrial fibrillation – data of the PREVEND cohort. PLoS ONE 12(2): e0171545. doi:10.1371/journal.pone.0171545

**Editor:** Florian Kronenberg, Medizinische Universität Innsbruck, AUSTRIA

**Received:** May 10, 2016

**Accepted:** January 23, 2017

**Published:** February 3, 2017

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**Data Availability Statement:** The authors confirm that some access restrictions apply to the data underlying the manuscript. The raw data are available at the Trial Coordination Center of the University Medical Centre Groningen. Due to the ethical restrictions of the medical ethics committee of the University Medical Center Groningen, these data cannot be made publicly available in order to protect the confidentiality of the study subjects' private information. However, researchers can contact Prof. Van der Harst of the PREVEND committee (email

address: [p.van.der.harst@umcg.nl](mailto:p.van.der.harst@umcg.nl)) for a request of the raw data.

**Funding:** The PREVEND study is supported by the Dutch Kidney Foundation (grant E0.13), the National Institutes of Health (grant 2R01LM010098), The Netherlands organization for health research and development (NWO-Groot grant 175.010.2007.006, ZonMw grant 90.700.441), and the Dutch Inter University Cardiology Institute Netherlands (ICIN), and the Netherlands Heart Foundation (grant NHS2010B280). Dr. M. Rienstra is supported by a grant from the Netherlands Organization for Scientific Research (Veni grant 016.136.055). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

## Introduction

Advancing age is one of the major risk factors for atrial fibrillation (AF). The prevalence of AF increases with advancing age, to approximately 8% in those older than 80 years.[1–3] Approximately 70% of the individuals with AF are 65 to 80 years of age.[4,5] At all ages, AF is more frequently present in men than in women.[6–8] However, the exact reasons behind the influence of age on AF are not completely understood.

Telomere length shortens with advancing age and is considered to be a marker of biological aging.[9] Telomeres are DNA-protein complexes located at the ends of chromosomes, and are essential structures preventing DNA degradation. The ability of protection and maintenance of chromosomal stability becomes increasingly limited as a result of repeated cell division. When cell proliferation, senescence, or apoptosis occurs, this results in loss of telomere length.[10–12] Shortening of telomere length is observed in an inconsistent linear decline throughout life, and is considered to be a marker for biological aging.[9] Telomere length shortening is associated with cardiovascular diseases, including atherosclerosis, left ventricular hypertrophy and heart failure.[12]

Interestingly, AF is more common in men than in women, and men have shorter telomere lengths than women.[13] These observations may suggest a relation between telomere length and incident AF. However, in previous studies no relation has been found.[14] We hypothesize that shorter telomere length is associated with incident AF, as mechanism underlying the observed association between age and sex with incident AF. We studied the association of telomere length and incident AF in healthy individuals included in the community-based Dutch Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort.

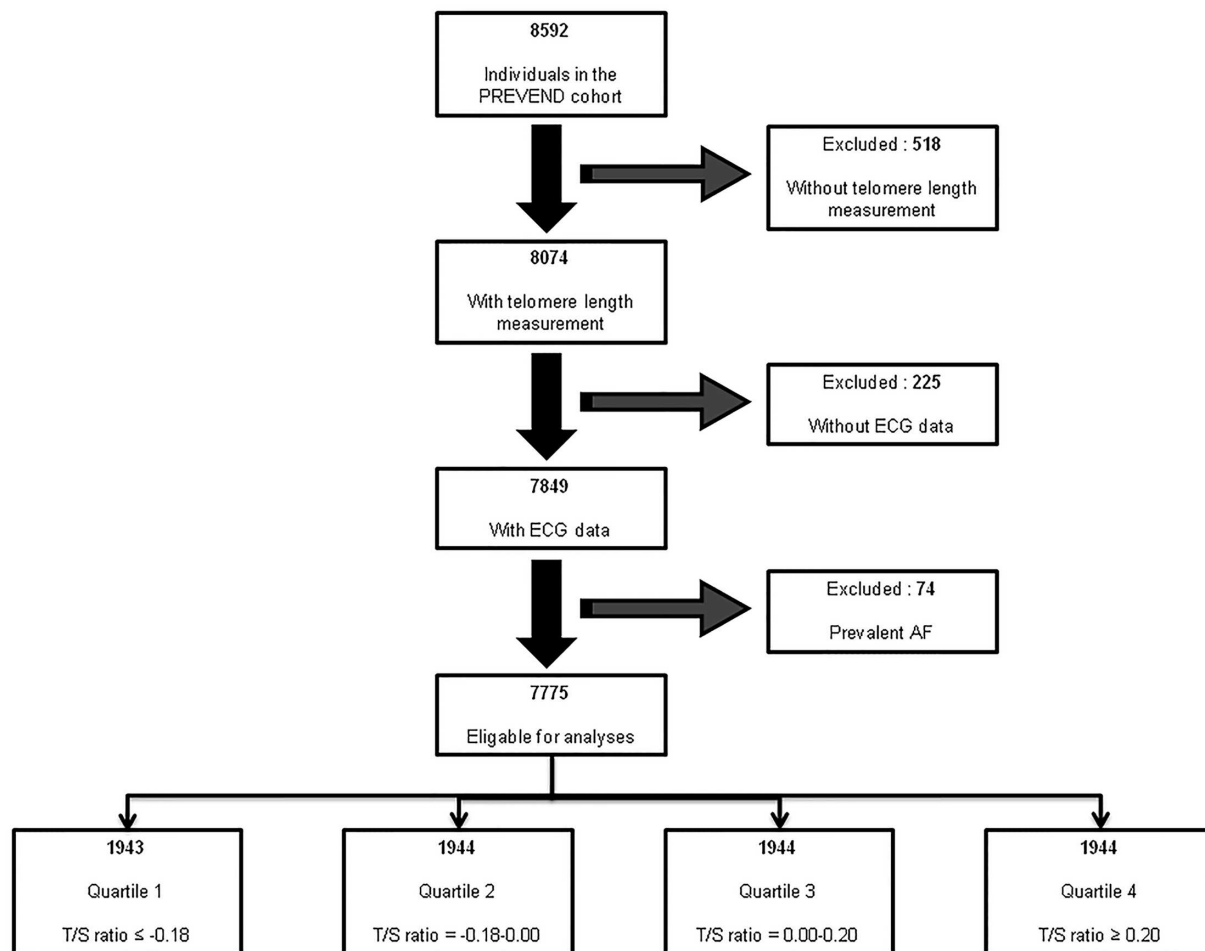
## Materials and methods

### Population

The association of telomere length and incident AF was examined in the PREVEND study, a community-based cohort, founded in 1997 in Groningen, the Netherlands. A detailed description of PREVEND has been published. In total, 8592 individuals were included in the PREVEND study. AF assessment has been described previously.[3] In brief, at each three-year interval study visit ECGs, blood and urine samples were collected. In addition, ECGs made at hospital visits and hospital admission between the study visits were screened for AF. We excluded individuals with no telomere length information ( $n = 518$ ), individuals without ECG data ( $n = 225$ ), and individuals with prevalent AF ( $n = 74$ ) from analysis (Fig 1). The medical Ethics Committee of the University Medical Center Groningen approved the PREVEND study, and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

### Covariate definitions

Systolic and diastolic blood pressure was calculated using an automatic Dinamap XL Model 9300 series device as the mean of the last two measurements of the two visits. Hypertension was defined as self-reported use of anti-hypertensive treatment, or as systolic blood pressure  $>140$  mmHg, and diastolic blood pressure  $>90$  mmHg. Antihypertensive treatment was defined as angiotensin receptor blockers, angiotensin converting enzyme inhibitors, calcium antagonists or diuretics. Body mass index (BMI) was defined as the ratio of weight to height squared ( $\text{kg/m}^2$ ), and obesity as a BMI  $>30$   $\text{kg/m}^2$ . A fasting plasma glucose  $\geq 7.0$  mmol/L (126 mg/dL), or a non-fasting plasma glucose  $\geq 11.1$  mmol/L, or use of anti-diabetic treatment was appointed as diabetes mellitus. Total serum cholesterol of  $>6.5$  mmol/L (251 mg/dl) or a



**Fig 1. Exclusions for present study.**

doi:10.1371/journal.pone.0171545.g001

serum cholesterol of  $>5.0$  mmol/L (193 mg/dL) if a history of myocardial infarction was present or use of lipid lowering treatment was defined as hypercholesterolemia. Nicotine use in the last 5 years was described as smoking. Myocardial infarction or stroke was defined as participant-reported hospitalization for at least 3 days for this condition. Previously, an expert panel ascertained heart failure in detail.[15] The glomerular filtration rate (eGFR) was measured using the simplified modification of diet formula. Measurements of highly sensitive C-reactive protein (highly sensitive CRP) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were described previously.[16,17]

### Follow-up

Duration of follow-up was defined as the time between the baseline screening visit to first AF, the last contact date (end of the third PREVEND follow-up visit), or death. The latest last contact date was December 31, 2010.

### Leukocyte telomere length measurement

DNA was drawn from full blood using a standard DNA extraction kit (Qiaamp, Qiagen, Venlo, the Netherlands). Mean leukocyte telomere length was determined from blood taken at the



**Table 1. Baseline characteristics.**

Clinical profile	Total (n = 7775)	Incident AF (n = 367)	No AF (n = 7408)	P-value
Telomere length	0.00 (-0.18–0.20)	-0.03 (-0.21–0.13)	0.00 (-0.18–0.21)	0.013
Age-years	49±13	62±9	48±12	<0.001
Male sex	3872 (50%)	254 (69%)	3618 (49%)	<0.001
BMI-kg/m2	25.6 (23.1–28.4)	27.5 (25.2–29.9)	25.5 (23.1–28.3)	<0.001
Obesity	1210 (16%)	90 (25%)	1120 (15%)	<0.001
Systolic blood pressure-mmHg	129±20	143±22	128±20	<0.001
Diastolic blood pressure-mmHg	74±10	79±9	74±10	<0.001
Heart rate-bpm	69±10	67±10	69±10	<0.001
Hypertension	2093 (27%)	198(54%)	1895 (26%)	<0.001
Heart failure	17 (0.2%)	6 (1.6%)	11 (0.1%)	0.002
Diabetes mellitus	285 (4%)	35 (10%)	250 (3%)	<0.001
Smoking	3442 (45%)	143 (39%)	3299 (45%)	0.040
Hypercholesterolemia	333 (4%)	42 (12%)	291 (4%)	<0.001
Glucose lowering treatment	107 (2%)	11 (3%)	96 (2%)	0.020
Lipid lowering treatment	314 (5%)	41 (13%)	273 (4%)	<0.001
Myocardial infarction	228 (3%)	50 (14%)	178 (2%)	<0.001
Stroke	54 (0.7%)	8 (2.2%)	46 (0.6%)	0.003
Antihypertensive treatment	1036 (16%)	131 (41%)	905 (15%)	<0.001
PR-interval-ms	158 (143–172)	168 (153–187)	158 (143–172)	<0.001
Biomarker profile	Total (n = 7775)	Incident AF (n = 367)	No AF (n = 7408)	P-value
eGFR-ml/min/1.73m2	80.9±14.5	76.6±15.7	81.1±14.4	<0.001
Creatinine-umol/L	82.0 (73.0–92.0)	88.0 (77.0–98.0)	82.0 (73.0–91.0)	<0.001
NTpro-BNP-ng/L	37.3 (16.6–72.6)	86.3 (40.8–195.5)	35.7 (16.0–69.0)	<0.001
hs-CRP -mg/L	1.27 (0.55–2.94)	1.97 (0.94–3.66)	1.25 (0.54–2.89)	<0.001
Glucose-mmol/L	4.9±1.2	5.4±1.7	4.9±1.2	<0.001

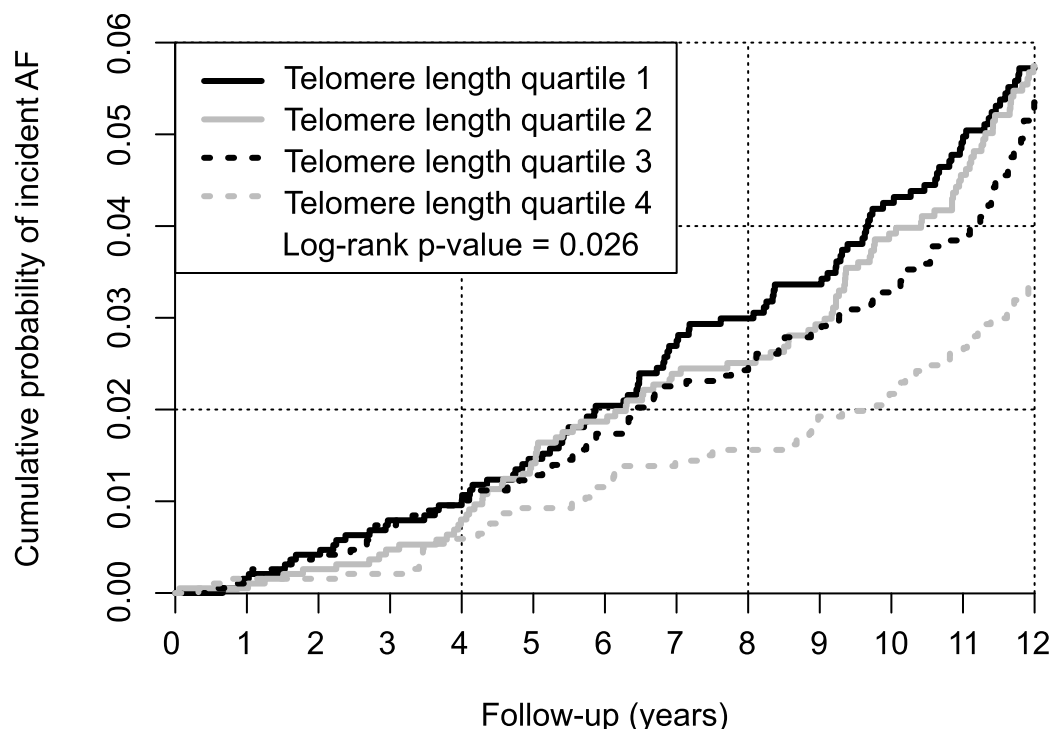
Data is expressed as mean ± standard deviation, median (interquartile range) or numbers (%). Telomere lengths are divided in quartiles. **Abbreviations:** AF = atrial fibrillation, BMI = body mass index, eGFR = estimate glomerular filtration rate, hs-CRP = highly sensitive C-reactive protein, NT pro-BNP = N-terminal prohormone of brain natriuretic peptide.

doi:10.1371/journal.pone.0171545.t001

Individuals that developed AF were older, more often men, and had more cardiovascular risk factors and diseases, except for smoking, which was less common in those with incident AF (39% versus 45%,  $p = 0.04$ ). The most common cardiovascular risk factor in those with incident AF was hypertension (54%), obesity (25%), hypercholesterolemia (12%), and diabetes (10%).

## Telomere length and risk of atrial fibrillation

Telomere length was shorter in individuals developing incident AF compared to those without AF ( $p = 0.013$ ). When discretizing individuals into quartiles based on the telomere length, the number of individuals with incident AF was inversely related to the telomere length. In the longest telomere length quartile 68 (3.5%) individuals developed incident AF, where 100 (5.1%) individuals developed AF in the shortest telomere length quartile ( $p = 0.032$ ) (Fig 2, S1 Table). Telomere length was associated with incident AF in the second shortest telomere length quartile using the longest telomere length quartile as reference (hazard ratio 1.64; 95% confidence interval 1.02–2.66;  $p = 0.043$ ). After age-adjusted analysis the relation between short telomere length and incident AF was no longer significant. In additional regression models, adjustments for sex and cardiovascular risk factors were performed, but in none of the



**Fig 2. Cumulative incidence curve of incident AF, by quartiles of leukocyte telomere length.** Quartile 1 represents the shortest telomere length group. Quartile 4 represents the longest telomere length group.

doi:10.1371/journal.pone.0171545.g002

models a significant association was found (Table 2). We performed secondary analyses with age as the time-variable, which led to similar findings as our primary analyses.

### Effect modification of AF risk factors by telomere length

As secondary analyses, we studied effect modification of age, sex and other conventional AF risk factors. For most AF risk factors, we found a significant interaction with telomere length for the association with incident AF (S2 Table). For age, systolic blood pressure, BMI and heart failure the interaction with telomere length was  $>1$ , meaning that the presence of the risk factor weakens or counteracts the association between shorter telomere length and incident AF, or, equivalently that decrease in telomere length weakens or counteracts the association between incident AF and the risk factor. For male sex and stroke, the interaction with telomere length was  $<1$ , meaning that the presence of the risk factor strengthens the association between shorter telomere length and incident AF, or, equivalently that decrease in telomere length strengthens the association between incident AF and the risk factor. For myocardial infarction, and antihypertensive treatment no effect modification was found.

In addition, we performed stratified analyses in three age categories  $<50$  years, 50–65 years, and  $>65$  years. In the age category  $>65$  years, the third shortest telomere length quartile was associated with AF (hazard ratio 2.70; 95% confidence interval 1.09–6.66,  $p$ -value = 0.031). No association between telomere length and incident AF was found in any of the other age categories or for one of the other telomere length quartiles (S3 Table).

In the analysis stratified for sex, the second shortest telomere length quartile in men was associated with AF (hazard ratio 2.06; 95% confidence interval 1.13–3.76,  $p$ -value = 0.018). No association between telomere length and incident AF was found for women (S4 Table).



**Table 2. Telomere length in quartiles and risk of incident AF.**

	Telomere quartiles	HR(95% CI)	P-value
Unadjusted model	1. ( $\leq -0.18$ )	1.43(0.85–2.39)	0.181
	2. ( $-0.18$ – $0.00$ )	1.64(1.02–2.66)	0.043
	3. ( $0.00$ – $0.20$ )	1.32(0.83–2.09)	0.245
	4. ( $\geq 0.20$ )	1(reference)	
Age-adjusted model	1. ( $\leq -0.18$ )	0.69(0.44–1.10)	0.123
	2. ( $-0.18$ – $0.00$ )	1.03(0.67–1.60)	0.882
	3. ( $0.00$ – $0.20$ )	1.05(0.68–1.64)	0.820
	4. ( $\geq 0.20$ )	1(reference)	
Sex-adjusted model	1. ( $\leq -0.18$ )	1.38(0.82–2.32)	0.221
	2. ( $-0.18$ – $0.00$ )	1.61(0.99–2.59)	0.053
	3. ( $0.00$ – $0.20$ )	1.31(0.82–2.07)	0.258
	4. ( $\geq 0.20$ )	1(reference)	
Model adjusted for sex, BMI, antihypertensive treatment, myocardial infarction, stroke, heart failure, PR-interval	1. ( $\leq -0.18$ )	0.84(0.50–1.42)	0.517
	2. ( $-0.18$ – $0.00$ )	1.19(0.72–1.96)	0.490
	3. ( $0.00$ – $0.20$ )	1.19(0.71–1.98)	0.509
	4. ( $\geq 0.20$ )	1(reference)	

Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference (quartile 4). The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0.

**Abbreviations:** AF = atrial fibrillation, BMI = body mass index, CI = confidence interval, HR = Hazard ratio.

doi:10.1371/journal.pone.0171545.t002

## Discussion

One of the main risk factors of AF is advancing age. We therefore hypothesized an association between shorter telomere length and AF. However, the observed association between leukocyte telomere length and AF diminished when age was included in the model. Effect modification by most conventional risk factors was present, suggestive for a role of telomere length via AF risk factors in development of AF, although more research is needed.

### Telomere length, biological ageing and incident atrial fibrillation

Biological ageing is marked by shortening of telomere length, and short telomere length is associated with several age-related cardiovascular diseases, including atherosclerosis, left ventricular hypertrophy and heart failure.[12] Telomere length has been suggested to be susceptible to age-related diseases.[20] Our finding that telomere length is not independently associated with AF is consistent with an analysis of the Cardiovascular Health Study investigators, who also, found no association between telomere length and incident AF.[14] The Cardiovascular Health Study analysis was restricted to individuals >65 years in the United States, and sample size was modest (1639 individuals). We included 7775 individuals with a wide age range between 29 and 74 years. Nevertheless, results of both studies were consistent. Reasons for the absence of an independent relation between telomere length and incident AF can be the following. First, telomere length was measured in leukocytes, and this may not be fully representative of telomere length in atrial cells, [14] although several previous studies found a positive relation between telomere length in leukocytes and AF-related cardiovascular diseases.[14, 21–24] Differentiated cardiomyocytes undergo limited cell division after embryogenesis, and telomere length shortening in left atrial cardiomyocytes and dividing leukocytes may be different. However, telomere biology in left atrial cardiomyocytes is



thought to play a role in the initiation of AF.[14] Premature apoptosis of cardiomyocytes resulting from loss of telomere length may contribute to fibrous replacement, and subsequently facilitate electrical chaotic activity (re-entry) that occurs in AF.[25–27] Secondly, other risk factors may contribute more than telomere length to the association of ageing and AF. Chronological ageing has been proven a risk factor of incident AF after adjustments for presence of cardiovascular risk factors and diseases, in numerous community-based cohorts.[3,14,28,29] With ageing stiffening of the left ventricle, and subsequent more diastolic dysfunction, atrial enlargement may occur, which may set the stage for AF.[30,31] Also sinus node dysfunction, and increase in premature atrial beats with ageing may be other mechanisms increasing the susceptibility for AF.[32–35] Thirdly, since we found modification by most conventional risk factors of the relation between telomere length and incident AF, it is possible that telomere length is not directly and independently related to development of AF, but via AF risk factors. Whether the presence of an AF risk factor itself influences the relation between telomere length and incident AF, or whether telomere length influences the relation between an AF risk factor and incident AF cannot be determined on present data. The further clarify the importance of effect modification of most AF risk factors by telomere length more studies are warranted, that compare patients with the same risk factors but different telomere lengths. Finally, other not-measured or subclinical risk factors and diseases potentially present in individuals at risk for AF may have influenced the association between ageing and development of AF (residual confounding).

## Strengths and limitations

Strengths of our analysis are the well-characterized large cohort, and the prospective design. The study also had several limitations. First, AF ascertainment is insensitive to asymptomatic paroxysms of AF, so asymptomatic AF may have been overlooked. And no information on temporal patterns of AF was available. Secondly, undetected age-related (cardiovascular) diseases may have influenced the results (residual confounding). Thirdly, telomere length was measured in leukocytes, and not in atrial cardiomyocytes. Fourthly, telomere length is measured at one time-point, and telomere length declines throughout life and the rate of telomere length attrition could affect the incidence of AF.[9] Fifthly, long term storage may have had an impact on telomere length, but the time frame of data collection was relatively small in our study, thus large differences in telomere length between sampling dates seem unlikely. All DNA samples were stored at -80°C, and no defrosting cycles were done before analyses. Lastly, results cannot be extended to other ethnicities, telomere lengths are longer in Afro-Americans for example, since the majority of individuals included were of European ancestry.

## Future perspectives

Despite our study results, telomere biology may be of importance in the development of AF-risk factors and AF-associated cardiovascular outcomes, such as stroke, heart failure, death, and the progression of AF. Effect modification by AF-risk factors was present, suggestive for a role of telomere length via AF risk factors in development of AF, although more research is needed. Furthermore, in a cross-sectional analysis of patients with and without a history of AF included in the Intermountain Heart Collaborative Study differences were found in telomere lengths according to paroxysmal, persistent and permanent AF.[36] So, the progression of AF may be associated with a higher rate of shortening of telomeres. Further investigation is needed to study telomere biology in the setting of AF.

## Conclusions

Shorter leukocyte telomere length is not associated with incident AF in the community-based cohort of PREVEND. Effect modification by most conventional risk factors was present, suggestive for a role of telomere length via AF risk factors in development of AF, although more research is needed.

## Supporting information

**S1 Table. Baseline characteristics according to quartiles.** Data is expressed as mean  $\pm$  standard deviation, median (interquartile range) or numbers (%). Telomere lengths are divided in quartiles. Abbreviations: AF = atrial fibrillation, BMI = body mass index, eGFR = estimate glomerular filtration rate, hs-CRP = highly sensitive C-reactive protein, NT pro-BNP = N-terminal prohormone of brain natriuretic peptide.  
(PDF)

**S2 Table. Association of telomere length and incident AF, modified by AF risk factor.** <sup>a</sup> No significant interaction means that no effect modification was present for that AF risk factor. An interaction hazard ratio  $<1$  means that the presence of the risk factor strengthens the association between shorter telomere length and incident AF, or, equivalently that a decrease in telomere length strengthens the association between incident AF and the risk factor. Similarly, an interaction hazard ratio  $>1$  means that the presence of the risk factor weakens or counteracts the association between shorter telomere length and incident AF, or, equivalently that an increase in telomere length intensifies the association between incident AF and the risk factor. Abbreviation: BMI = body mass index. <sup>a</sup> Model includes AF risk factor, telomere length and the interaction-term of both. <sup>b</sup> All continuous covariates were centered around their means.  
(PDF)

**S3 Table. Cox regression models by age categories.** Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference. The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = atrial fibrillation, CI = confidence interval, HR = Hazard ratio.  
(PDF)

**S4 Table. Cox regression models by sex.** Hazard ratios are calculated for quartiles with shorter telomeres with the longest quartile as reference. The telomere length is the ratio of telomere expression divided by reference gene and is standardized per standard deviation. Log transformed T/S ratios were centered around 0. Abbreviations: AF = atrial fibrillation, CI = confidence interval, HR = Hazard ratio.  
(PDF)

## Author contributions

**Conceptualization:** MR.

**Data curation:** MR BG.

**Formal analysis:** BG.

**Funding acquisition:** MR PVDH.

**Investigation:** JES MR.

**Methodology:** JES BG MR.

**Project administration:** JES.

**Resources:** MR PVDH.

**Software:** BG.

**Supervision:** MR.

**Validation:** BG MR.

**Writing – original draft:** JES MR.

**Writing – review & editing:** BG ICVG PVDH MR.

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